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# Bioleaching of heavy metals from spent household batteries using *Acidithiobacillus ferrooxidans*: Statistical evaluation and optimization

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ABSTRACT

In this study, spent batteries were selected from municipal waste for bioleaching and extraction of their heavy metals. The bioleaching of nickel, cadmium, and cobalt from Ni–Cd and NiMH batteries was done using *Acidithiobacillus ferrooxidans*, a resistant strain used for bioleaching. The adaptation process was successful and the solid-to-liquid ratio reached 10 g/L (battery powder weight/volume of medium). A Box–Behnken design of response surface methodology (RSM) was used to investigate the effects of initial pH, powder size, and initial $Fe^{3+}$ concentration on the percentage of metals recovered. The proposed statistical method was used to accurately evaluate the interactions of the factors and their effects on the recovery efficiency of nickel, cadmium and cobalt during bioleaching. Under the specified conditions, up to 99% recovery was observed for each metal, confirming that *A. ferrooxidans* is an effective toxin resistant microorganism for bio-recovery of heavy metals. Decreasing the initial pH and particle size and increasing the initial $Fe^{3+}$ concentration led to maximum recovery for nickel and cobalt; while the optimum condition for cadmium was different for Ni and Co. To maximize simultaneous extraction of three metals, the optimum value for initial pH, particle size and initial $Fe^{3+}$ concentration were obtained 1, 62 μm and 9.7 g/L, respectively. Under these conditions, the recoveries predicted by the software (Design Expert version 7.1.4) were 85.6% for Ni, 66.1% for Cd, and 90.6% for Co. These results were confirmed using a verification experiment at optimum condition, which calculated 87%, 67%, and 93.7% for Ni, Cd and Co, respectively. Due to the distinct chemical characteristics of Cd in contrast with Ni and Co, its recovery in the optimal condition for simultaneous recovery was decreased; in the other words; Cd recovery was higher when it was the sole target for extraction.

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## 1. Introduction

Nowadays people all around the world are using different types of electrical devices which lots of them work with battery, like cordless telephones, flashlights, radios and cell phones, to name a few. Thus, the battery disposal in municipal solid waste has become a serious problem for many countries. The high rate of battery usage and the fact that they contain significant amounts of toxic heavy metals make it crucial to find an appropriate solution for their disposal. Recovery of these metals allows their recycling and decreases environmental risks associated with disposal of batteries in landfills.

Some solid waste is an even richer source of minerals than mineral ore, making recovery of these waste metals valuable as secondary sources of metals. Spent batteries are also classified as a secondary source of valuable metals [1]. Studies have shown that household batteries contain more than 50% of the cadmium found in solid municipal waste in the USA [2]. Developed countries have enacted strict rules for the collection of electrical waste and have developed strategies to recover them [2]. The recovery methods are divided into conventional and new bio-processing methods.

Pyrometallurgical and hydrometallurgical processes are common conventional methods for metal recovery, but they difficult to control, cause secondary pollution, and are expensive and risky [3]. The mining industry has shown bioleaching to be an effective method of metal extraction. In contrast to the other conventional methods, bioleaching consumes less energy and is environmentally friendly, but does require complicated technology [3]. It is based on the ability of microorganisms to convert insoluble solid compounds into soluble and extractable forms.

Studies have attempted to determine how much bioleaching is practical when extracting heavy metals from different sources, especially metals existing in solid waste, such as the byproducts of metallurgy industries, spent catalysts, spent batteries and other wastes [1]. Bioleaching is also an effective method for soil

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remediation and recovery of heavy metals from contaminated soil [4]. This process has been used to extract Al, W, Mo, V, Co, Cd and Ni from spent refinery catalysts, spent batteries as well as Cu from copper convertor slag and printed circuit boards [1–5]. High percentages of recovery from blast furnaces have been reported for Fe and other heavy metals using bacterial leaching. Good recovery rates of Cr from tannery sludge (87%) and Mn from electrolytic manganese residue (93%) have been reported using sulfur oxidizing bacteria [5]. Bioleaching of Au, Pt and Pd from electronic waste and Cd recovery from fly ash from municipal solid waste has also been studied [1].

Promising recovery yields for bio-recovery of metals from spent batteries have been found for Co and Li from spent lithium-ion batteries, Zn and Mn from spent Zn–Mn batteries and Ni, Cd and Co from Ni–Cd batteries [3,6–12]. Among all kinds of batteries, Ni–Cd batteries are represented as the most toxic ones and are classified as hazardous waste. These results confirm the practicality of bioleaching in the recovery of heavy metals from spent batteries.

As one of the most toxic heavy metals, a few studies have been done on cadmium bio-recovery, so in this research the Ni–Cd batteries were chosen as cadmium containing electrical waste. Having nickel in Ni–Cd batteries, it was decided to mix two kinds of batteries with almost the same heavy metals composition, thus adding NiMH batteries and studied the bioleaching process for mixed anode and cathode materials. In this way the results of this study would be helpful and practical for scaling up to an industrial process since the primary separation of solid waste into individual battery type and materials of cathode and anode will face difficulties.

Having a mixture of different phases (solid powder and the culture itself) we had to consider the probable interactions of process parameters on the results. The best approach is designing of experiments. Because of the chemical interaction between metallic compounds and other solution particles which mainly caused by iron ions (iron sulfate used as the energy source of the bacteria) initial $Fe^{3+}$ concentration was considered as one of the factors to investigate its effects on recovery percentage.

In this study for rechargeable Ni–Cd and NiMH spent batteries, the dominant phase of powder, as determined by initial analysis, are the metallic hydroxides which are considerable both for chemical reaction in the medium and studying particle size effects on the bio-recovery yield because of their special crystalloid structures which have not been studied for spent batteries yet. For *Acidithiobacillus ferrooxidans*, as an acidophilic generous, initial pH was chosen as the third factor to complete the set of effective factors for experimental design.

In brief the present study evaluated the effects of particle size, initial pH, and initial $Fe^{3+}$ concentration on the recovery efficiency of nickel, cadmium and cobalt in the bioleaching process of spent household batteries using the Box–Behnken design of response surface methodology. Determination of possible interactions between the variables as well as optimal conditions for simultaneous extraction of Ni, Cd and Co was also studied.

## 2. Materials and methods

### 2.1. Preparation of battery powder

Spent Ni–Cd and NiMH household batteries were chosen as the second-hand source of the heavy metals to be recovered. First, sample powder was prepared by manually disassembling several Ni–Cd and NiMH (AA 1.2 V) spent batteries. Because of the presence of both nickel and cadmium compounds at the anode and cathode poles, the pole materials were mixed and powdered in an electric blender (model: Moulinex, AR1044, 180 W) to obtain a fine homogenous mixture. This method side-stepped the difficult dismantling of the batteries into two discrete parts and is more practical on an industrial scale.

### 2.2. Microorganism and the growth medium

The mesophilic *A. ferrooxidans* strain was chosen for bioleaching. This strain is commonly used for bioleaching because of its ability for ferrous ion oxidation to ferric as a powerful oxidizing agent in a non-contact mechanism [12]. *A. ferrooxidans* (PTCC 1647) was provided from the Iranian Research Organization for Science and Technology (IROST) as a native strain. The 9 K medium comprising $(NH_4)_2SO_4$, 3.0 g/L; KCl, 0.1 g/L; $K_2HPO_4$, 0.5 g/L; $MgSO_4{\cdot}7H_2O$, 0.5 g/L; $Ca(NO_3)_2$, 0.01 g/L; $FeSO_4{\cdot}7H_2O$, 44.22 g/L (99.9%, Merck) was used to obtain a bacterial growth curve for the fresh strain. A maximum cell density of $10^9$ cells/mL gained at 30 °C and 160 rpm for the shaking incubator.

### 2.3. Adaptation of bacteria to battery powder

Bacteria adaptation to the powder was done by serial passaging until the pulp density reached 10 g powder per 1 L medium [9]. Adaptation was initiated by adding 0.1 g of powder to 100 mL of culture with an initial cell density of $10^7$ cells/mL. The next step was inoculation of 90 mL of fresh medium with 10 mL of adapted bacterial culture and the addition of a further 0.1 g of powder. In each step the proper time for passaging was determined by the cell count. When the cell count reached to a fixed magnitude, considered as the stationary phase of the adapted bacteria, we shifted to the next stage. For the adapted *A. ferrooxidans*, the cell count in stationary phase was nearly in the order of $10^8$ cells/mL. Each step took about 8 days to complete. Serial passage was continued to a pulp density of 10 g/L. Bacterial resistance was monitored at a pulp density of 1.1 g powder/L. The bacterial count did not reach to the acceptable value and decreased to near zero after 2 days, thus a pulp density of 10 g/L was considered to signify bacteria tolerance threshold.

### 2.4. Analytical methods and apparatus

Inductively coupled plasma mass spectrometry (ICP-MS) analysis (model: Varian/Agilent; 710 ICP-OES) was used to determine the composition of the powder used to calculate metal recovery. X-ray diffraction (XRD) (model: Rigaku; Miniflex II desktop) was used to identify the component phases of the initial sample powder and the final leaching residue. The XRD results aided analysis of the chemical reactions during bio-recovery and determining of the effect of bioleaching parameters on the process.

Surface morphology of the battery powder was determined by scanning electron microscopy (SEM) (model: Philips XL30) before and after bioleaching to determine the demolition of the metallic compounds. The chemical composition of the final solution was determined by atomic absorption spectrometry (AAS) (model: Varian 240FS) to compute the percentage of recovery of metals with having initial metals amounts in the powder.

Because of the high concentration of $FeSO_4{\cdot}7H_2O$ (44.22 g/L) in the medium, the measurement of ferrous and ferric ions is important to assessment of the success of bioleaching. 5-Sulfosalicylic acid (SSA) testing was used for ferric ion measurement to evaluate the bacterial activity in ferrous ion oxidation to ferric using a standard diagram of absorbance [13]. After 20 days SSA was performed to calculate the amount of ferric ions produced. Eh for all values was measured using an Eh meter (model: Metrohm, 691) to evaluate the oxidative reductive specifications of the solution. To analyze the acidity variation, pH of each flask was measured using a pH meter (model: p25, IsTek).

The number of free bacteria was estimated using a Neubauer chamber with a depth of 0.1 mm and an area of 0.0025 $mm^2$. An optical microscope (Olympus) with 1000× magnification was used for this purpose.

### 2.5. Statistical approach

#### 2.5.1. Design of experiments (DoE)

Several parameters affect the bio-recovery of heavy metals. Therefore a Box–Behnken design, as a statistical method with a quadratic polynomial model, was used to examine the effects of the parameters and interactions between variables on the metals extraction. In a Box–Behnken design, each numeric factor is varied over three levels. In this study, particle size, initial pH, and $Fe^{3+}$ concentration were the factors determined. Box–Behnken allows a decrease in test runs from 27 ($3^3$) to 17, so it features economy of experimentation [14]. The Box–Behnken design also avoids all corner points and star points and allows fitting of the quadratic model [15,16]. 17 Experiments were designed by Box–Behnken method using equation of $k^2 + k + c_p$, where $k$ is the number of factors and $c_p$ is the number of center points which equals to 5.

After completion of adaption up to a pulp density of 10 g/L, 17, samples were prepared according to the specified conditions. The experiments were done in 17 Erlenmeyer flasks using different powder particle sizes, initial pH values, and $Fe^{3+}$ concentrations (Table 2). The Erlenmeyer flasks were placed into the rotary shaker incubator of 160 rpm at 30 °C with an initial cell density of $10^7$ cells/mL (cells of adapted bacteria per volume of medium).

According to the coexisting of three heavy metals of nickel, cadmium and cobalt compounds in the battery powder, we optimized the process conditions to maximize the simultaneous metals extraction by choosing the goal of maximizing all three metals recovery percent for the software. In this way we looked for gaining the maximum recovery yield for the two more expensive heavy metals (Co: 26.4 $/kg, Ni: 13.6 $/kg and Cd 2 $/kg, [17]) and at the same time dissolving maximum amount of the most toxic one, Cd, for the environmental considerations.

**Table 1**
Chemical composition of the sample powder using ICP.

| Element | Weight percent (%) |
|---|---|
| Ni | 40 |
| Cd | 13.6 |
| Co | 3.6 |
| La | 3.5 |
| Ce | 1.7 |
| Zn | 0.9 |
| Mn | 1 |
| Al | 0.4 |
| Fe | 0.1 |

#### 2.5.2. Selected bioleaching parameters

The most important variables involved in bioleaching were temperature, pulp density, energy source or substrate concentration, pH, and particle size. Adaptation was done at a constant temperature of 30 °C and a pulp density of 10 g of battery powder per liter of culture. Therefore the experiments were designed and accomplished at constant pulp density of 10 g/L for each sample, while levels were specified for three other factors in bioleaching assessment.

*2.5.2.1. Initial ferric ion concentration.* Since ferric ion is an oxidizing agent, it was considered as one factor in the bio-recovery of metals [18]. $Fe_2(SO_4)_3$ was first added to the medium to release the ferric ions to stimulate the oxidation reduction reaction. The inhibitory effect of $Fe^{3+}$ ions on bacteria at concentrations greater than 17 g/L of $Fe_2(SO_4)_3$ restricted [19], so the amount used was chosen 0, 5 and 10 g/L for the low, center and high levels, respectively.

*2.5.2.2. Initial pH. A. ferrooxidans* is an acidophilic genus that prefers a pH of 1–2.5. The initial pH range was between 1 and 2.6 with the center point fixed at 1.8 to allow accurate measurement by the pH meter. The pH values of 1, 1.8, and 2.6 were selected to determine the optimum initial pH.

*2.5.2.3. Particle size.* Mass transfer is a challenge in chemical engineering process also in bioleaching. Contact surface is one of the factor affect the mass transfer. In bioleaching processes the tension between particles surface caused by the solid pulp may damage and lyse the cells wall. By decreasing the particle size, this tension and at the same time the mass transfer increases because of the increasing of contact surface. These two parameters should be balanced by choosing the right mesh size for the battery powder. The sample powder was sifted through three sieves with different mesh sizes, using a horizontally oscillating movement, so that the powder particle size was divided into three ranges. A nominal diameter was defined for each range (Table 3). We used a size range between 37 and 149 μm, thus the mean size of 93 μm

**Table 2**
Box–Behnken design based on three significant parameters and the metals recovery as the responses.

| Run order | Experimental factors | | | Metal recovery (%) | | |
|---|---|---|---|---|---|---|
| | Initial pH | Initial $Fe^{3+}$ conc. (g/L) | Size (μm) | Ni | Cd | Co |
| 1 | 1.80 | 0.00 | 37.00 | 70.0 | 41.3 | 58.3 |
| 2 | 1.00 | 5.00 | 149.00 | 45.4 | 72.3 | 48.0 |
| 3 | 1.00 | 10.00 | 93.00 | 64.4 | 73.7 | 70.3 |
| 4 | 1.00 | 0.00 | 93.00 | 78.2 | 85.0 | 43.1 |
| 5 | 1.00 | 5.00 | 37.00 | 100.0 | 46.9 | 100.0 |
| 6 | 1.80 | 5.00 | 93.00 | 50.6 | 71.4 | 58.6 |
| 7 | 2.60 | 0.00 | 93.00 | 74.2 | 100.0 | 48.5 |
| 8 | 2.60 | 5.00 | 149.00 | 41.0 | 92.3 | 41.0 |
| 9 | 1.80 | 10.00 | 37.00 | 79.3 | 42.3 | 84.9 |
| 10 | 1.80 | 5.00 | 93.00 | 73.6 | 99.7 | 74.1 |
| 11 | 1.80 | 5.00 | 93.00 | 57.6 | 81.2 | 57.4 |
| 12 | 1.80 | 5.00 | 93.00 | 61.1 | 80.4 | 60.8 |
| 13 | 1.80 | 10.00 | 149.00 | 42.4 | 75.1 | 50.5 |
| 14 | 2.60 | 5.00 | 37.00 | 77.8 | 43.8 | 70.0 |
| 15 | 1.80 | 0.00 | 149.00 | 51.9 | 78.7 | 38.4 |
| 16 | 2.60 | 10.00 | 93.00 | 44.8 | 63.7 | 39.9 |
| 17 | 1.80 | 5.00 | 93.00 | 49.6 | 66.3 | 48.7 |

**Table 3**
Sieve mesh sizes and battery powder characteristics.

| Mesh | Nominal sieve opening (μm) | Particle size (μm) | Nominal diameter |
|---|---|---|---|
| 400 | 37 | $37 < d_1 < 74$ | $d_1$ |
| 200 | 74 | $74 < d_2 < 149$ | $d_2$ |
| 100 | 149 | $d_3 > 149$ | $d_3$ |

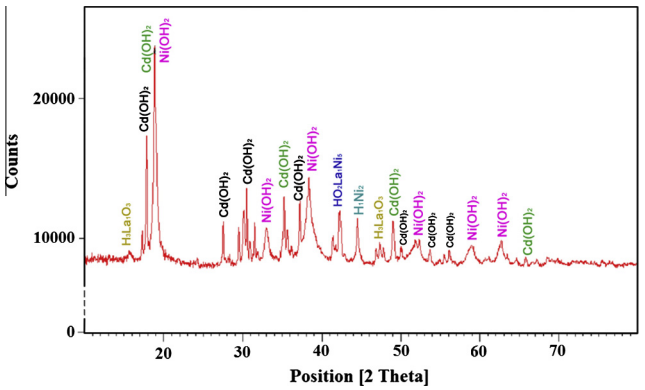


**Fig. 1.** XRD pattern of initial battery powder material.

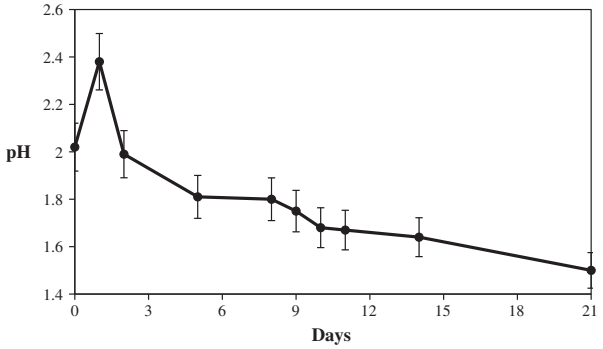


**Fig. 2.** pH variation during adaption process in a pulp density of 10 g/L.

(nominal diameter of $d_2$) was chosen as the central point for designed experiments (Table 2).

## 3. Results and discussion

### 3.1. Sample powder analysis

Results of initial battery powder analysis are shown in Table 1 and Fig. 1. Ni, Cd and Co were identified as the main elements of the powder by ICP analysis. XRD analysis indicated that metallic hydroxides were the predominant phases of the powder. NiMH batteries are a source of lanthanum (M) compound, as indicated in the XRD results. Co compound was not detected by XRD because of its fine structure, which made it hard to detect [3].

### 3.2. pH variation during bacterial adaptation

In each step of adaptation, a variation in pH was observed. Fig. 2 shows that, initially, the pH increased because the initial dissolution of metallic hydroxides and oxidation of ferrous ions by the *A. ferrooxidans* consumed protons Eq. (1):

$$Fe^{2+} + 1/4O_2 + H^+ \xrightarrow{A.\ ferooxidans} Fe^{3+} + 1/2H_2O \quad (1)$$

As adaptation continued, ferric ion hydrolysis released protons, which decreased pH [9]:

$$Fe^{3+} + H_2O \iff Fe(OH)^{2+} + H^+ \quad (2)$$

$$Fe^{3+} + 2H_2O \iff Fe(OH)_2^+ + 2H^+ \quad (3)$$

$$Fe^{3+} + 3H_2O \iff Fe(OH)_3 + 3H^+ \quad (4)$$

### 3.3. Bioleaching experiments and statistical modeling

The difference between initial and final ferric ion concentrations was calculated for each sample (Table 4), dash mark placed for minus magnitudes (samples with precipitation formation). In samples with an initial pH of 1, more ferric ions dissolved in the medium; at the higher initial pH values, according to Fig. 3 jarosite precipitate was the dominant form. This can be explained by $Fe^{2+}/Fe^{3+}$ – Eh–pH diagrams [20]. For samples with jarosite precipitation, the total pH decreased as a result of the jarosite formation reaction [21]:

$$K^+ + 5OH^- + 3Fe^{3+} + 2SO_4^{2-} + H_2O \rightarrow KFe_3(SO_4)_2(OH)_6 + H^+ \quad (5)$$

SEM was used to survey the surface morphology of the powder before and after bioleaching treatment. Fig. 4(a) shows primary structures of the anode and cathode material. The granular structures belong to cadmium compounds while the spherical particles are nickel hydroxide [3] that dissipated after bio-treatment, as observed in Fig. 4(b). According to XRD results for leached residue (Fig. 3), the powdery structure of the residue (Fig. 4(b)) contain jarosite, formed during bioleaching process also it can be observed that there may be no metal hydroxides remained in leached residue as it is not detected by XRD analysis.

For all 17 samples, AAS analysis was conducted to measure the dissolved nickel, cadmium and cobalt, which are listed in Table 2. Recovery percent for the three metals, as the responses of the bioleaching process, were calculated, and analyzing based on these values was accomplished by the software.

With a confidence threshold of 0.05 (confidence level of 95%) and the model *p*-values of 0.049, 0.0399 and 0.011 for the three responses, the model was annotated significant in ANOVA (Table 5). A second-order polynomial equation for recovery of every metal was obtained as follows:

$$\begin{aligned} \text{Ni}\ (\%) = {} & 144.04589 - 46.08925\ (\text{pH}) + 1.87863\ (Fe^{3+}) \\ & - 0.51132\ (\text{Size}) - 0.97188\ (\text{pH}) \times (Fe^{3+}) \\ & + 0.099281\ (\text{pH}) \times (\text{Size}) - 0.016755\ (Fe^{3+}) \\ & \times (\text{Size}) + 9.40638\ (\text{pH})^2 + 0.034259\ (Fe^{3+})^2 \\ & + 0.000482174\ (\text{Size})^2 \end{aligned} \quad (6)$$

$$\begin{aligned} \text{Cd}\ (\%) = {} & 15.79358 - 15.64293\ (\text{pH}) + 2.68151\ (Fe^{3+}) \\ & + 0.21383\ (\text{Size}) - 1.56127\ (\text{pH}) \times (Fe^{3+}) \\ & + 0.12892\ (\text{pH}) \times (\text{Size}) - 0.00411435\ (Fe^{3+}) \\ & \times (\text{Size}) + 4.14224\ (\text{pH})^2 - 0.074285\ (Fe^{3+})^2 \\ & - 0.00593284\ (\text{Size})^2 \end{aligned} \quad (7)$$

$$\begin{aligned} \text{Co}\ (\%) = {} & 95.73479 - 2.79116\ (\text{pH}) + 9.90232\ (Fe^{3+}) \\ & - 0.83615\ (\text{Size}) + 0.12794\ (\text{pH}) \times (\text{Size}) \\ & - 0.012972\ (Fe^{3+}) \times (\text{Size}) - 2.23499\ (\text{pH}) \\ & \times (Fe^{3+}) - 2.11649\ (\text{pH})^2 - 0.32423\ (Fe^{3+})^2 \\ & + 0.00198229\ (\text{Size})^2 \end{aligned} \quad (8)$$

**Table 4**
Variations in produced $Fe^{3+}$, pH and Eh the designed experiments samples after 20 days.

| Run order | Initial $Fe^{3+}$ conc. (g/L) | Produced $Fe^{3+}$ (g/L) | Initial pH | ΔpH | Eh (mV) |
|---|---|---|---|---|---|
| 1 | 0 | 1.42 | 1.8 | 0.1 | 522 |
| 2 | 5 | 7.65 | 1 | 0.3 | 516 |
| 3 | 10 | 5.6 | 1 | 0.3 | 529 |
| 4 | 0 | 9.85 | 1 | 0.5 | 546 |
| 5 | 5 | 9.6 | 1 | 0.3 | 540 |
| 6 | 5 | 0 | 1.8 | 1.3 | 516 |
| 7 | 0 | 0.99 | 2.6 | 2 | 517 |
| 8 | 5 | – | 2.6 | 1.5 | 539 |
| 9 | 10 | – | 1.8 | 1.4 | 538 |
| 10 | 5 | – | 1.8 | 1.4 | 519 |
| 11 | 5 | – | 1.8 | 1.4 | 510 |
| 12 | 5 | – | 1.8 | 1.4 | 517 |
| 13 | 10 | – | 1.8 | 1.3 | 504 |
| 14 | 5 | – | 2.6 | 1.6 | 526 |
| 15 | 0 | 2.15 | 1.8 | 1.5 | 488 |
| 16 | 10 | – | 2.6 | 1.5 | 512 |
| 17 | 5 | – | 1.8 | 1.4 | 514 |

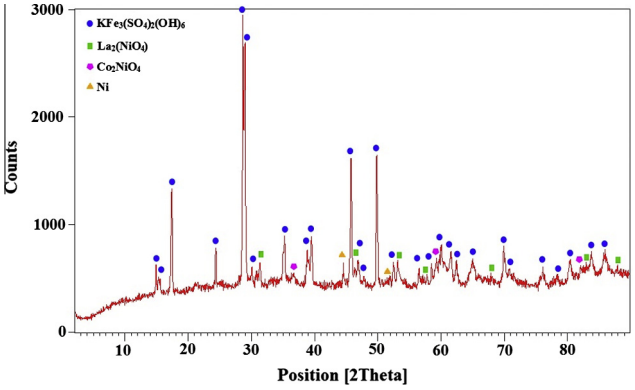


**Fig. 3.** XRD pattern of battery powder residual after bioleaching.

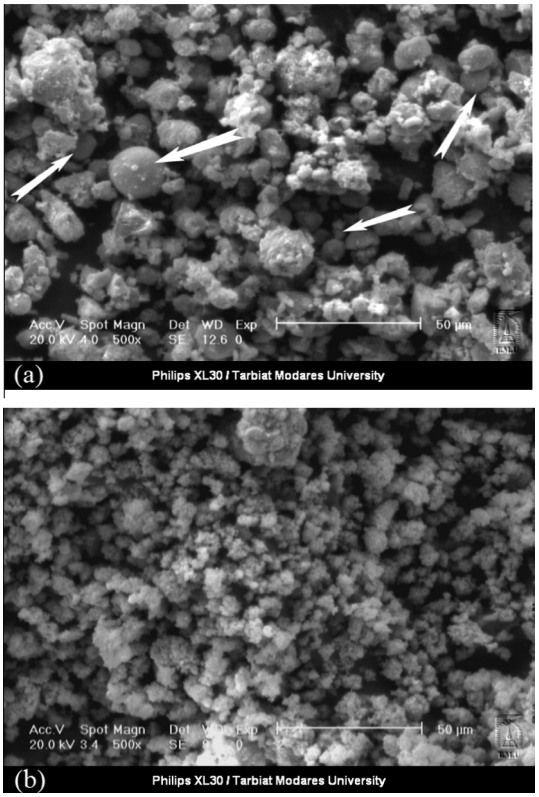


**Fig. 4.** SEM for: (a) the initial battery powder and (b) solid residue after 20 days bioleaching (magnification: 500×).

According to the *p*-values of process variables reported in ANOVA table and also the factors coefficients of Eqs. (6)–(8), the size factor with the smallest *p*-value and greatest coefficient was most effective. *p*-Values for the lack of fit were not significant for the responses, confirming the fitness of the models (Table 5). Predicted versus experimental values (Fig. 5(a–c)) confirm that, for the three responses, the data points were split evenly at the 45° line.

The interaction plot for Co is presented in Fig. 6. The non-parallel lines indicate that the effect of initial ferric concentration was dependent on the level of pH and vice versa. The slopes were significant, indicating that the interaction of these two parameters is considerable for Co.

Ni and Co were found in the finest size of powder, which resulted in higher recovery efficiency; Cd produced better results at the larger mesh size (Fig. 7(a–f)). Metallic hydroxides have a general formula of $(M(OH)_y)_n$ where M stands for Ni, Co, and Cd and *n* is the number of metallic hydroxide compounds in a crystal structure. The value for *n* is greater for Cd than for Ni and Co [22]; this indicates that, for cadmium hydroxides, the accessible surface area increased as the size of the powder increased, resulting in higher recovery efficiencies for cadmium. It seems that for larger Cd compounds the accessibility of Ni and Co hydroxides reduced, thus particle size decreasing, increased Ni and Co recovery percentage, while recovery yield increased by size increasing for Cd.

The trends of variation for Co resembled that for Ni for all factors; for Ni and Co, the effect of pH was similar to that for size on recovery. For Cd, however, a decrease in pH increased recovery efficiency (Fig. 7(c)). Nickel and cobalt hydroxides are soluble at acidic pH values of 1–1.5, but cadmium hydroxide better dissolves at a pH range of 2.5–9 [23].

Ferric ions increased nickel and cobalt hydroxide recovery. Based on Eqs. (3) and (4), ferric reducing reactions lead to proton formation which increases Ni and Co recoveries. According to the discharging–recharging reactions of Ni–Cd battery, initial NiO(OH) turns to $Ni(OH)_2$. For the dissolved battery powder, $Ni(OH)_2$ reacts with proton ions:

$$Ni(OH)_2 + 2H^+ \rightarrow Ni^{2+} + 2H_2O \quad (9)$$

As shown in Fig. 7(b), for Ni in pH values less than 1.5, pH variation is more effective on recovery yields than that of ferric ions concentration, while for cobalt, best recovery efficiencies occurred in pH values less than 1.5 and ferric ions concentrations more than 5 g/L.

At higher initial concentrations of ferric ions due to jarosite formation and its adsorption ability it appeared that iron ions excluded cadmium ions from the solution in a precipitation reaction (chemical adsorption) leading to formation of $FeOCdOH_2^+$ sediment [9,24] and decreased cadmium hydroxide extraction efficiency. Reaction equations for Cd compound are as follow:

$$Cd + 2Fe^{3+} \rightarrow 2Fe^{2+} + Cd^{2+} \quad (10)$$

$$Cd(OH)_2 + 2H^+ \rightarrow Cd^{2+} + 2H_2O \quad (11)$$

Velgosová et al. [9] investigated the effect of ferric ion concentration on chemical leaching and bioleaching of Cd (no report data for nickel and cobalt recovery) [9]. Their results for Cd showed a variation in pH throughout bioleaching confirmed the suitability of pH values greater than 2 for cadmium hydroxide solution, as

**Table 5**
ANOVA table for response surface models applied.

| Responses (recovery %) | $R^2$ | Model $p$-value | | Lack of fit $p$-value | | Factor $p$-value | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Initial pH | Initial $Fe^{3+}$ conc. | Particle size |
| Ni | 0.82 | 0.049 | Significant | 0.3636 | Not significant | 0.135 | 0.187 | 0.0017 |
| Cd | 0.84 | 0.04 | Significant | 0.6701 | Not significant | 0.52 | 0.166 | 0.003 |
| Co | 0.9 | 0.011 | Significant | 0.6358 | Not significant | 0.0346 | 0.0465 | 0.0007 |

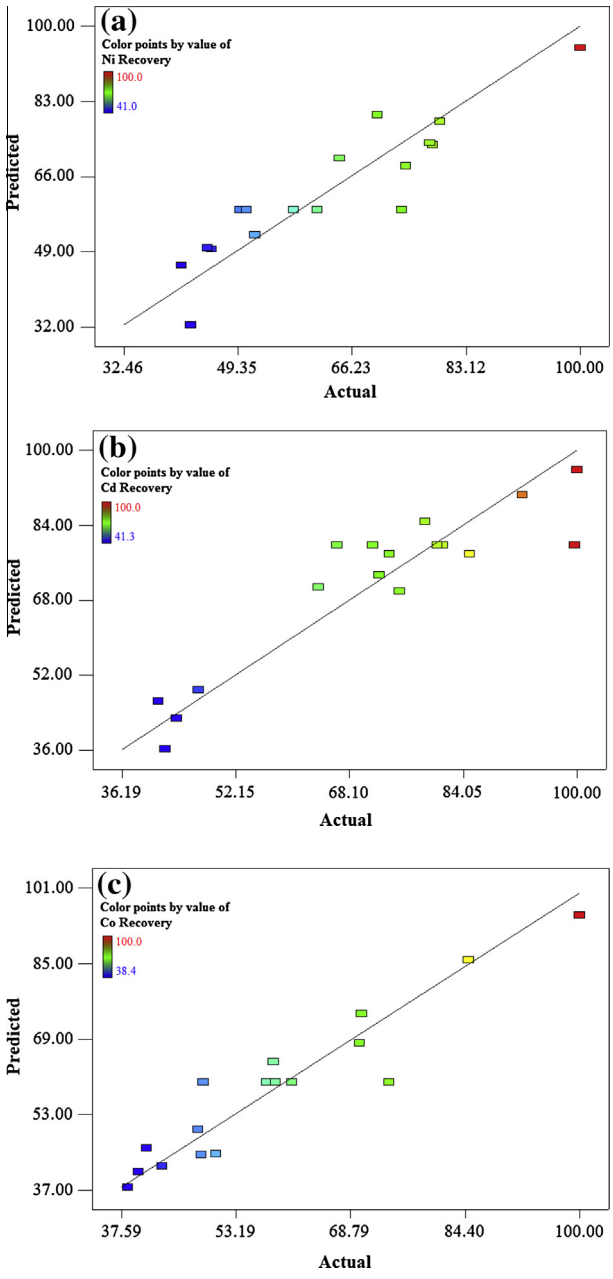


**Fig. 5.** Predicted versus actual plots for: (a) Ni recovery (%), (b) Cd recovery (%) and (c) Co recovery (%). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

shown in the present study. According to their results in leaching process by $Fe_2(SO_4)_3$, initial ferric concentration of about 100 g/L is needed to reach 100% recovery for cadmium while in bioleaching process as we did in this study with maximum initial ferric ion concentration of about 10 g/L, recovery yield reached to 100% for

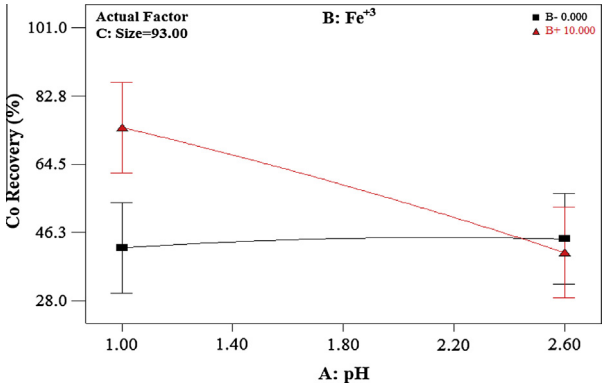


**Fig. 6.** Interactions of initial pH and ferric concentration parameters at a constant size value for cobalt.

nickel and cobalt and for cadmium as discussed before, this amount reduces to 0 g/L initial ferric ion concentration. Also Velgosova et al. studied the Ni bio-recovery from spent Ni–Cd batteries. They only reached less than 50% recovery yield for nickel [25]. Zhao et al. [3] examined spent Ni–Cd bio-recovery by sewage sludge. The whole bio-process completed in about 40 days in a two stage continuous flow system whereas the diagnostics analysis for samples of designed experiments of this study indicated the completion of bioleaching process in about only 15 days. Also they found the trend of variation for nickel and cobalt extraction resembled each other as we discussed for Ni and Co.

Nanwe Zue et al. recovered nickel and cadmium from spent Ni–Cd batteries using sewage sludge, a mixed culture of *A. ferrooxidans* and *Acidithiobacillus thiooxidans*. The process was operated on three stages and at different RTBs (residence time of the sludge in bioreactor). The best recovery yields for cadmium and nickel reported as 81.7% and 71.6% respectively (no report for cobalt) and did not achieve full bio-recovery.

### 3.4. Optimization

Optimization defined targets for three responses simultaneously using predefined conditions. The behavior of Cd was distinct from the other metals, thus, the process was optimized to maximize synchronous extraction of all three metals. The optimum condition was modeled at the lowest initial pH (pH = 1), maximum initial ferric ion concentration (9.7 g/L) and a moderate particle size (62 μm) and produced recovery yields of 85.6%, 66.1% and 90.6% for Ni, Cd and Co, respectively.

### 3.5. Confirmation testing

To test the validity of the optimized condition, an experiment was done in the optimal condition. According to Table 6 the experimental results were within high and low predicted values in 95% confidence intervals. It means that the experimental and model responses were almost similar or near each other, confirming that the model successfully predicts the experimental response.

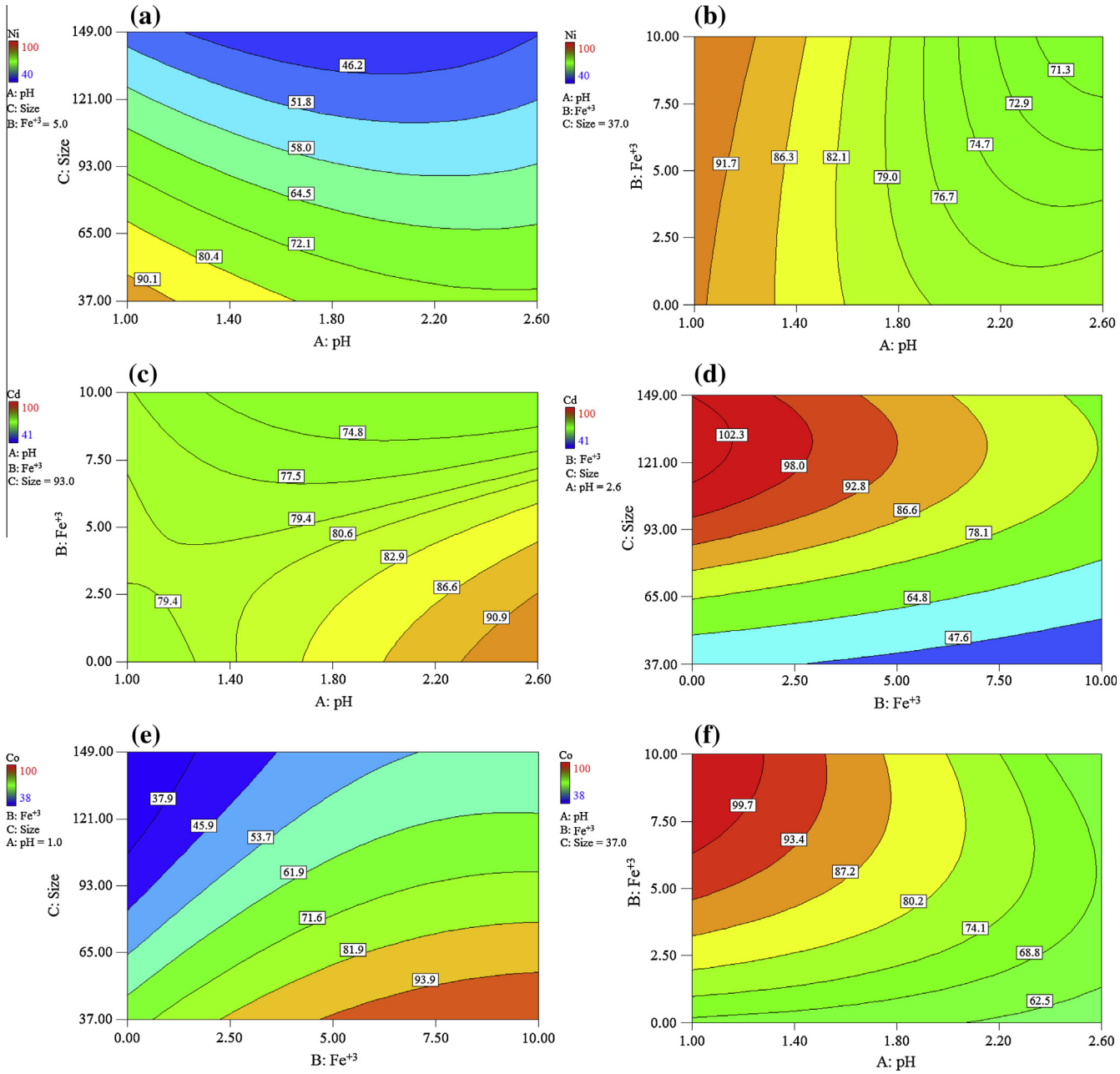


**Fig. 7.** (a) and (b) contour plots of Ni recovery (%) at constant initial ferric concentration and particle size, respectively; (c) and (d) contour plots of Cd recovery (%) at constant particle size and initial pH, respectively; (e) and (f) contour plots of Co recovery (%) at constant initial pH and particle size, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Table 6**
Point prediction and validation of the responses at the optimal point.

| Responses (recovery %) | Prediction (recovery %) | 95% CI low (recovery %) | 95% CI high (recovery %) | Confirmation experiment (recovery %) |
|---|---|---|---|---|
| Ni | 85.6 | 63.37 | 100 | 87 |
| Cd | 66.2 | 42 | 90.42 | 67 |
| Co | 90.6 | 72.87 | 100 | 93.7 |

## 4. Conclusion

In this research, for each individual metal bioleaching conditions, recovery was up to 99%, proving the resistance of *A. ferrooxidans* to heavy metal toxicity. Bioleaching of solid waste containing metal compounds requires an experimental design to investigate the possibility of interaction effects of the process variables on metal recovery. The Box–Behnken design was able to determine the interactions and model the bioleaching at $R^2 > 0.9$, which indicates a high correlation of experimental responses with model predictions. It was found that increasing particle size and initial pH and decreasing ferric ion concentration for Cd resulted in the maximum recovery efficiency up to 99%. For nickel and cobalt hydroxides, however, the minimum particle size and initial pH and the maximum initial ferric ion concentration led to the highest bio-recovery efficiency. This indicates that, to achieve the highest simultaneous metals extraction, the process should be optimized at initial pH of 1, a mean particle size of 62 μm, and an initial ferric concentration of 9.7 g/L.

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